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Smoking and Varicocele Adversely affect Sperm Enzyme Profile as Evidenced by Alpha-Naphthyl Acetate Esterase, Acid Phosphatase and Alkaline Phosphatase Cytochemistry

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Abstract

Background: In addition to the other factors, varicocele is responsible for one third of male reproductive disorders. The incidence of varicocele ranges between 35-40% in men with primary infertility, increases to 80% in secondary infertility. Among adult males with varicoceles, 90% are present unilaterally on the left side while 10% are present bilaterally**.** Varicocelectomy has a beneficial effect on the improvement of semen quality and male fertility. Although the etiology of varicocele remains unclear, it reduces testicular blood renewal with a consequent accumulation of toxic substances. Thus, varicocele can potentiate the toxic effects of the genotoxic substances such as those found in cigarette smoke. Current human semen analysis is not sufficient to determine the fertility potential of a semen sample. In particular, there is insufficient information on the effects of varicocele and smoking on sperm enzymes. In this study, α-naphthyl acetate esterase, acid phosphatase and alkaline phosphatase activities of the human sperm were determined. Semen samples were obtained from sexually mature healthy non-smokers, non-smokers with varicocele, non-smokers with varicocelectomy, smokers without varicocele and smokers with varicocelectomy. Semen smears were prepared, air-dried, fixed. Enzyme cytochemically processed specimens were examined examined under a light microscope for positive reactions of the enzymes.

Results: The healthy non-smoking group group had the highest enzyme positivites of the investigated enzymes. The positivities significantly was lower in the non-smoker varicocele group and reached in its lowest level in the smoker varicocele group. Varicocelectomy significantly increased positivity percentages of the 3 enzymes. Nevertheless the increase in the with varicocelectomized smokers group was lower than the other groups.

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Conclusions: The enzyme cytochemical results of this study have revealed that smoking and varicocele signifcantly reduced the sperm counts and motility in accordance with the significant decreases in the alpha-naphthyl acetate esterase, acid and alkaline phosphatase positivity profiles of the samples. Although varicocelectomy provided significant improvement in the positivity profiles of the investigated enzymes, keeping smoking limited the positive effect of the varicocelectomy. Cytochemical demonstration of the sperm enzymes might be a useful tool in sperm quality evaluation.

Keywords: Acid Phosphatase**;** Alkaline Phosphatase**;** Alpha-Naphthyl Acetate Esterase; Semen**;** Sperm Enzymes

Abbreviations

ACP-ase: Acid phosphatase; ALP-ase: Alkaline phosphatase; ANAE: α-naphthyl acetate esterase; ICSI: Intracytoplasmic sperm injection; PBS: Phosphate buffer saline; ROS: Reactive oxygen species; RT: Room temperature; WHO: World Health Organization

Background

Infertility, classically defined as the inability to achieve pregnancy after 12 months of sexual relations well distributed throughout the menstrual cycle and without the use of contraceptives, affects around 15% of couples of reproductive age and the male partner is responsible for up to 50% of these cases [1].

Many adverse internal and external conditions, such as high testicular temperature, varicocele, nutrition, aging, environmental pollutants, infections, cryptorchidism, varicocele, drug addiction and smoking, have detrimental effects on male fertility through their unique pathophysiological mechanisms. The optimum temperature required for regular spermatogenesis to occur is approximately 35°C, and thermoregulation of the testicles is achieved by elongation of the musculus cremaster and heat exchange between the spermatic artery and the plexus pampiniformis using the countercurrent principle [2,3].

Varicocele (Curling varicocele), which is the abnormal dilatation of the efferent veins in the pampaniform plexus, is associated with alterered semen quality, decreased sperm functional integrity and seminal oxidative stress, diminished seminal plasma protein profiles which may cause the altered semen phenotype. Dilatation, stasis and high pressure in the plexus pampiniformis veins of the funiculus spermaticus are typical in the disease. Disposing of anatomical features, narrowing and obstruction of the veins are facilitating factors in the etiology of varicocele [3]. The left testis is more susceptible to varicocele. Since varicocele progressively damages testicular tissues over time, testicular development is regressed, spermatogenesis is disrupted and infertility develops. The increase of varicoele from 35% to 40% in men with primary infertility to 80% in men with secondary infertility, suggests that the disorder causes a progressive decline in male fertility [2]. Ligation of dilated veins greatly improves testicular functions including spermatogenesis and sperm quality in 80% of cases after the operation.

Varicocele can potentiate the toxic effects of environmental exposure to genotoxic substances such as cigarette smoke cecause that varicocele reduces testicular blood renewal resulting in accumulation of toxic substances [4]. Also, an association between oxidative stress and impaired sperm function in patients with varicocele has been evidenced by increased levels of reactive oxygen species(ROS) and reduced total antioxidant capacity [5].

It was long known that smoking has negative effects on sperm count and motility, as well as sperm morphology. Nicotine is the most important substance of approximately 4000 toxic substances found in smoke. In heavy smokers, semen volume, sperm count, sperm motility decrease and the rate of abnormal sperm increases. The most affected parameter in smokers is sperm motility, which determines sperm fertilization [6]. Smoking prevents these people from having children, even with assisted reproductive techniques. Because even in intracytoplasmic sperm injection (ICSI), motile sperm are also preferred in sperm se-

lection.

Traditional human semen analysis primarily determine morphology, concentration and motility of the sperm, because low sperm count, progressive forward motility and deterioration in morphology are considered evidence of fertility problems. Although these methods provide valuable results, they are not decisive in determining the fertility of the semen sample [7]. Therefore, intense efforts have been made to develop new laboratory tests that determine the fertilizing capacity of human sperm. More recent efforts to predict male fertility have focused on the ability of sperm to undergo the acrosome reaction. A positive correlation has been found between the frequency of induction of the acrosome reaction and fertility under in vitro conditions [8]. Larson and Miller [9] suggested that the acrosomal status of the sperm could be clearly determined within a few minutes using Coomassie blue staining.

A number of enzyme activities have been demonstrated in both sperm and seminal plasma of some mammalian species, including humans [10,11]. Previous researchers have used complex biochemical techniques to determine the amount and location of sperm enzymes [12]. Recently, cytochemical methods have begun to be used in research on sperm enzymes.

Most sperm enzymes are essential for the fertilization ability of sperm [13]. Acrosomal enzymes of sperm are mostly acidic hydrolytic enzymes that play an important role in sperm penetration during oocyte fertilization [13].

A water-soluble enzyme, acrosomal hyaluronidase is the first enzyme to leak from the acrosome into the seminal plasma and digests the hyaluronic acid between the cumulus oophoricus and corona radiata cells. Acid-phosphatase (ACP-ase) and alkaline-phosphatase (ALP-ase) were biochemically first detected in different pieces of human sperm by Wislocki [14]. Experiments by Allison and Hartree [15] revealed that ACP-ase is mainly located in the acrosome. Edvinsson et al. [10] also detected ACP-ase activity on the outer surface of the human sperm membrane, which was previously detected biochemically in seminal plasma. Non-specific esterase is mainly found in the middle piece of the mouse sperms [16]. Cholinesterase has been demonstrated in the head, midpiece and tail of bovine spermatozoa [17].

The cytochemical analysis of the enzymes in different pieces of sperm, especially in the sperm head and its acrosome, can provide useful clues in the evaluation of sperm quality. In the present study, the differences between the positivities of α-naphthyl acetate esterase (ANAE), ACP-ase and ALP-ase were determined in the semen samples of sexually mature and non-smokers without varicocele (healthy), non-smokers with varicocele, non-smokers with varicocelectomy, smokers without varicocele, and smokers with varicocelectomy.

Methods

Materials and Groups

In the study, the semen samples were taken from a total of 42 men with fertility problems, aged between 21-45 years, under the directives of the Ethics Committee of (Decision number: 2017/187 dated 07.6.2017). Semen donors were selected with their own consent among men accepted to the faculty's Andrology Laboratory. Donors who were smokers or nonsmokers had no medical history of chronic diseases such as serious urological infection and undescended testicles, diabetes, hypertension and rheumatism, with or without varicocele surgery.

After semen quality evaluation, semen samples were divided into 5 groups: non-smoker non-varicocele group, non-smoker varicocele group, non-smoker varicocelectomy group, smoker varicocele and smoker varicocelectomy groups.

Collecting and Quality Evaluation the Semen Samples

Semen samples were collected into sterile containers by masturbation after at least three days of sexual abstinence. For macroscopic examination, the samples were liquefied in an oven at 37°C for 15-60 minutes. After evaluating the viscosity, appearance, volume and pH of each sample, the samples were examined under a light microscope (CX41, Olympus, Japan) with a X20 objective.

Sperm Washing

For this purpose, 1 ml of fresh semen sample was diluted with 9 ml of phosphate buffer saline (0.1 M PBS, pH 7.4), and centrifuged at 800 G at room temperature (RT, 22°C) for 8 minutes. The process was repeated 3 times. After the third centrifugation, the sperm pellet was diluted with 2 ml of PBS. Semen smears were prepared, air-dried at RT, and stained (Spermac FP17S11, Minitube, Belgium).

Both macroscopic and microscopic semen quality were evaluated according to the fifth guideline of the World Health Organization (WHO) [18]. Sperms were counted and motility was determined using a hemocytometer. At least, 200 motile spermatozoa were counted in each sample, and motility data were expressed as the percentage of motile sperm of the total sperm count. Semen samples that were evaluated as azoospermic upon microscopic examination were excluded from the study. Appropriate smears were used in enzyme cytochemical procedures.

ANAE Cytochemistry

Semen smears were fixed in glutaraldehyde-acetone (pH 4.8) at −10°C for 5 min and then washed three times with distilled water. The incubation solution was prepared according to Çelik et al. [19]. The air-dried smears were kept in the incubation solution at 37°C in a controlled manner for 60 minutes. Upon formation of the reddish brown reaction product, the incubation was terminated, the slides were washed 3 times with distilled water, and the nuclei were stained with 2% methyl green (M8884, Sigma-Aldrich, Germany). Slides were coverslipped using synthetic resin.

ACP-ase Cytochemistry

Air-dried smears were fixed in a solution containing 10 ml of methyl alcohol, 60 ml of acetone, 30 ml of distilled water and 630 mg of citric acid at -10°C for 5 minutes. The enzymatic reaction was carried out by azo dye simultaneous coupling method [20]. The final pH of the incubation solution was adjusted to 5.0 with 1N NaOH solution and then filtered. The smears were kept in the solution at room temperature for 1 hour in a controlled manner. At the end of the incubation, nuclei were stained with 2% methyl green in acetate buffer (pH 4.8). Slides were coverslipped using Kaiser's gelatin.

ALP-ase Cytochemistry

ALP-ase activity was determined by the simultaneous azo-binding method according to Lojda et al. [21]. Briefly, smears were fixed in formal calcium at -10°C for 5 min, washed three times with distilled water, and air dried. The enzymatic reaction was confirmed by the azo dye simultaneous coupling method using sodium α-naphthyl phosphate (N7000, Sigma-Aldrich, Germany) as substrate and fast red TR (F6760, Sigma-Aldrich, Germany) as chromogen. The smears were incubated in the incubation solution in a controlled manner at room temperature for 10-15 minutes. When the reddish brown reaction product was formed, the incubation was terminated and the smears were washed 3 times with distilled water. The cell nuclei were stained with 2% methyl green in acetate buffer (pH 4.8), and slides were coverslipped using Kaiser's gelatin. All samples were examined with a Nikon Eclipse E-400 light microscope equipped with a DS-5M digital camera head and DS-L1 camera control unit (Nikon, Japan) with an X100 objective. In each smear, total sperm were counted until a count of 200 enzymatically positive sperm was reached, and the results were expressed as a percentage (%) of the total sperm count.

Statistical Analysis

The data were analyzed with the IBM SPSS (IBM Corp., Armonk, USA) software for Windows. Since the data obtained from the cytochemical representation of ANAE, ACP-ase, and ALP-ase were not normally distributed, the data were first analyzed by the Kruskal Wallis test. Mann Whitney U-test was used to compare the significance of the differences between the mean values of the groups. A value of $p< 0.05$ was considered statistically significant.

Results

Semen Quality of the Groups

Individual semen quality data of the donors are given in the table 1. Sperm concentration was highest in the non-smoking and non-varicocele groups, decreased significantly when smoking and varicocele coexisted, while the

smoking varicocele group had the lowest level.

Table 1: Semen quality parameters of the donors

Groups	Case numbers	Age (Year)	Sperm concentration(X10^6 cell/ml)	Total motility (%)	Progresive Motility (%)	Nonprogressive motility (%)	Sperm vitality (%)	Head abnormality (%)	Neck-middle piece abnormality (%)	Tail abnormality (%)
Non-smoker non-varicocele	1	45	80	70	51	19	76	86	14	16
	2	30	40	65	47	18	69	88	16	19
	3	34	80	70	55	15	73	89	16	23
	4	28	80	62	44	18	65	88	9	17
	5	55	52	69	56	13	74	89	14	9
	6	32	70	66	50	16	72	87	18	8
	7	28	20	65	45	20	69	89	14	19
Non-smoker varicole	8	32	28	79	61	18	82	87	17	13
	9	28	80	66	50	16	69	87	18	23
	10	20	60	70	53	17	75	89	13	18
	11	19	65	60	39	21	67	85	17	15
	12	28	55	76	60	16	79	89	13	21
	13	26	35	66	52	14	71	86	15	17
	14	28	54	78	60	18	82	89	9	18
	15	40	100	70	56	14	76	90	19	15
Non-smoker varicolectomy	16	42	60	67	54	13	68	87	10	15
	17	29	82	78	60	18	84	90	14	12
	18	27	30	70	53	17	74	88	13	22
	19	30	22	63	36	27	69	89	16	23
	20	37	25	68	52	16	72	85	18	21
	21	32	16	25	13	12	29	87	12	17
	22	24	60	70	55	15	73	90	15	18
	23	30	50	60	48	12	65	89	11	10
Smoker varicocele	24	29	80	70	52	18	76	86	19	21
	25	21	26	65	50	15	68	88	16	19
	26	18	32	68	50	18	71	89	18	18
	27	28	34	65	47	18	69	89	21	22
	28	24	72	60	42	18	63	90	13	16
	29	25	20	65	50	15	69	88	14	18
	30	35	60	65	52	13	71	89	12	19
	31	22	23	65	43	22	68	87	19	14
	32	31	28	70	52	18	75	90	15	27
	33	26	35	74	60	14	77	92	19	14
Smoker varicocelectomy	34	35	15	66	46	20	69	89	16	20
	35	30	16	69	50	19	73	89	16	22

	36	32	100	74	56	18	78	88	16	21
	37	28	24	71	54	17	76	87	11	12
	38	25	16	62	43	19	67	88	11	10
	39	35	20	50	35	15	54	89	11	18
	40	38	16	75	56	19	81	92	13	11
	41	36	20	60	40	20	66	89	22	7
	42	23	60	70	53	17	76	92	15	17

Varicocele alone caused a significant decrease in sperm concentration. Although varicocele surgery causes a significant increase in sperm concentration. Smoking weakened the positive effect of varicocele surgery (Figure 1).

The healthy non-smoker group had the highest sperm motility. Varicocele significantly reduced sperm motility. Varicocelectomy significantly increased sperm motility. However, smoking weakened the effectiveness of the varicocelectomy (Figure 1).

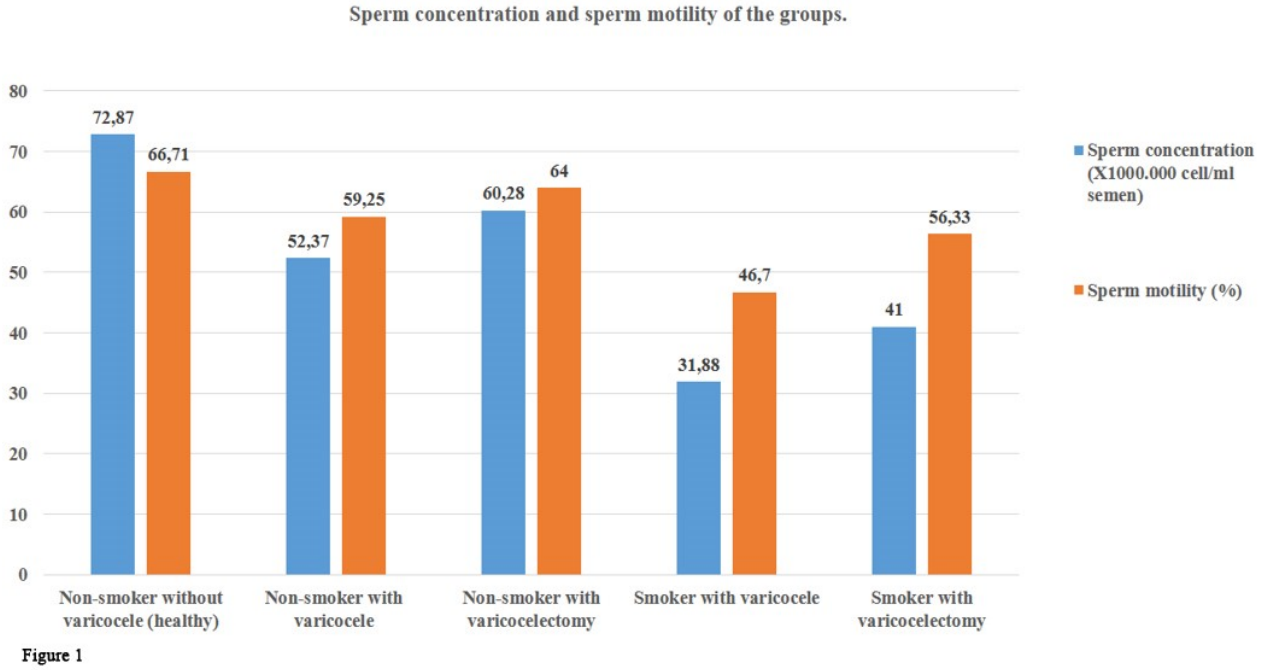

Figure 1: Sperm concentration and sperm motility of the groups

Morphological Evaluation of the ANAE Positivity

ANAE positivity was observed mainly in the head part of the sperm, behind the equator and close to the middle part, in the granular form and reddish-brown granules or bands adjacent to the cell membrane, and rarely in the form of bands localized in the middle part. Mononuclear somatic cells and semen droplets also gave strong ANAE positivity. The distribution and localization of the reaction product of the ANAE enzyme of the groups were quite similar (Figure 2).

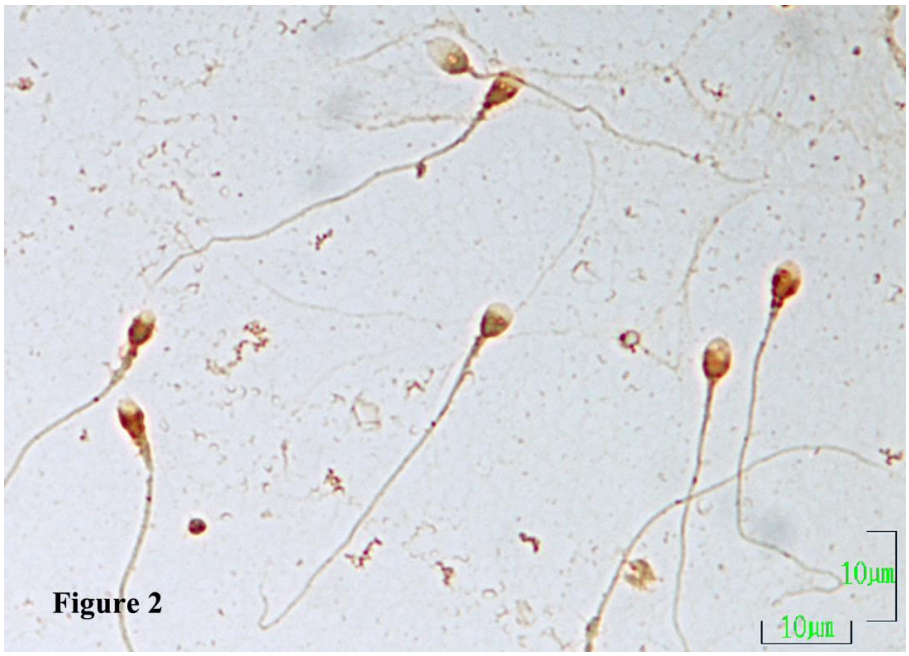

Figure 2: ANAE positive spermatozoa with different positivity patterns in the non-smoker varicocele group are seen. The reaction product is mainly located in the sperm head, behind the equator, and close to the middle piece, as granules and bands adjacent to the cell membrane, and rarely in the middle piece of the sperm. Semen droplets also gave strong ANAE positivity. ANAE demonstration. Magnification bar: 10 µm

Morphological Evaluation of the ACP-ase Positivity

ACP-ase positivity was observed in some samples as pale diffuse pinkish-red staining in the acrosome of the sperm and small granules stained dense pinkish-red in the same locations and in the middle and tail pieces. In most of the samples, the ACP-ase reaction product lcated in the middle piece of the sperm. The positivity was also observed in somatic cells and semen droplets. There was no difference between the groups in terms of distribution and localization of ACP-ase (Figure 3).

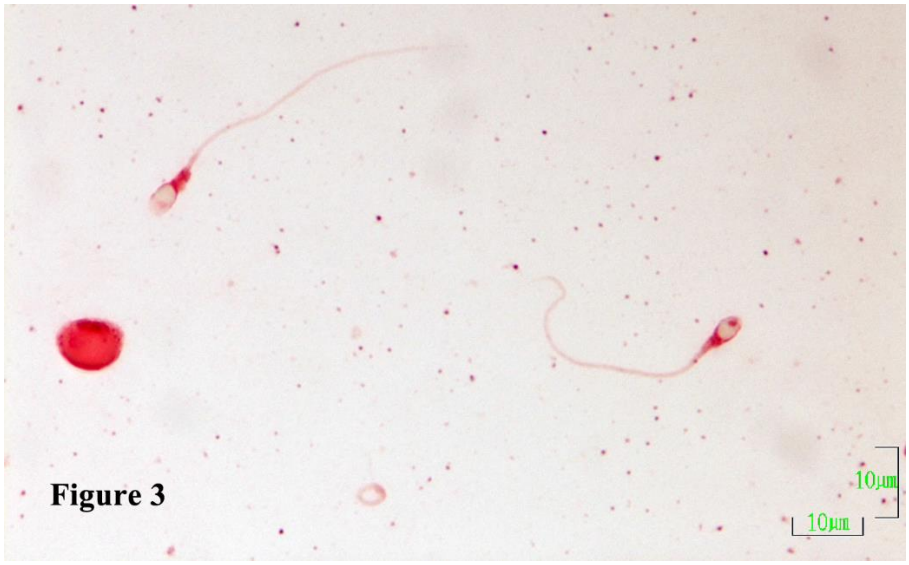

Figure 3: ACP-ase positive spermatozoa with different positivity patterns in the smoker varicocelectomy group are seen. The pinkish-red reaction products are located mainly as a pale diffuse staining in the acrosome, stronger in the middle, and weakly stained product in the tail of the sperm. Somatic cells and semen droplets also gave a positive reaction. ACP-ase demonstration. Magnification bar: 10 µm.

Morphological Evaluation of the ALP-ase Positivity

ALP-ase positivity was observed as reddish-brown granules located predominantly in the head and neck regions of the sperm. No significant difference was observed between the ALP-ase enzyme positivity patterns of the groups (Figure 4).

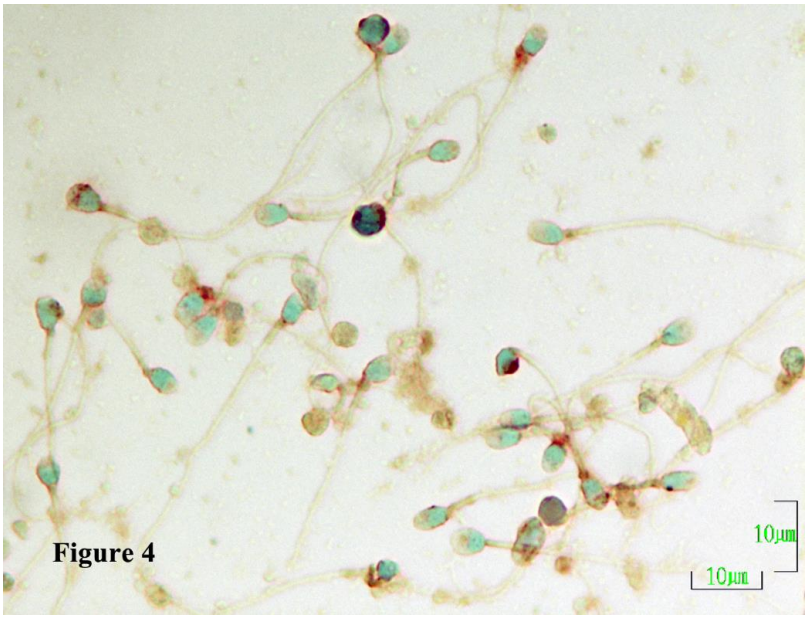

Figure 4: ALP-ase positive spermatozoa in the non-smoker varicocele group. ALP-ase reaction products are reddish-brown small granules, which are mainly located in the head and neck pieces of the spermatozoon. ALP-ase demonstration. Magnification bar: 10 µm

Enzyme Positivity Frequency Results

Significant individual differences were observed in ANAE, ACP-ase, and ALP-ase positivity. The mean ANAE, ACP-ase, and ALP-ase positivity of each of the groups were quite similar and displayed similar changes (Figure 5).

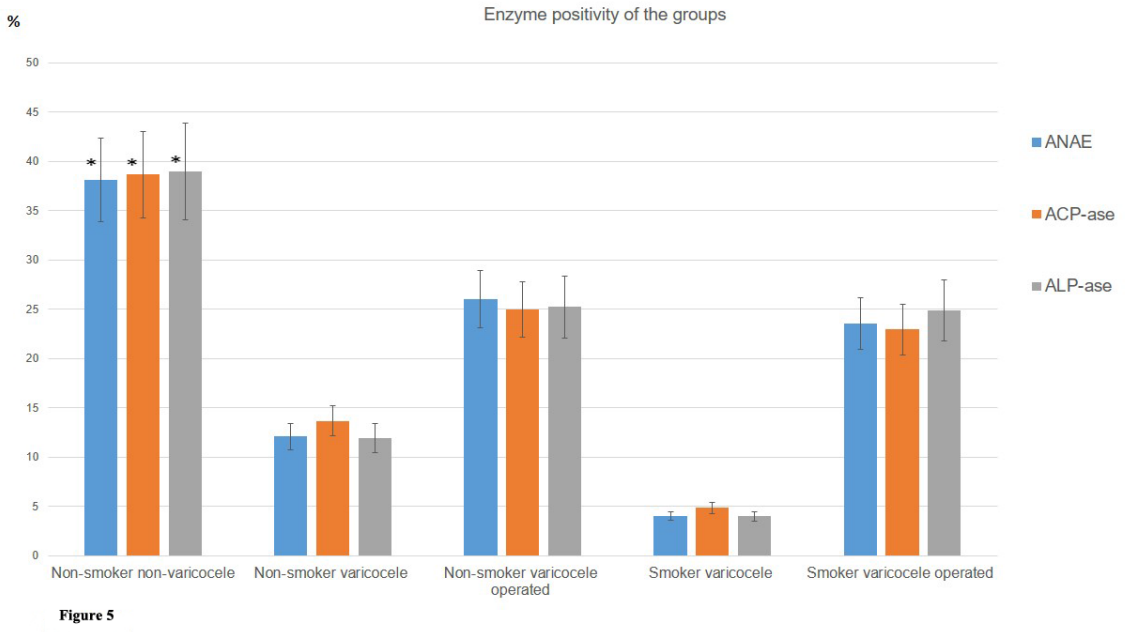

Figure 5: Enzyme positivity of the groups

ANAE positivity was highest (38.11%) in the non-smoking and non-varicocele group. ANAE positivity decreased significantly (P<0.05) in the non-smoker varicocele group (12.06%). The lowest (4.00%) ANAE positivity was observed in the smoker group with varicocele (Figure 5). ANAE positivity was significantly higher in the non-smoking varicocelectomy group (26.00%). In the smoker varicocelectomy group, ANAE positivity increased significantly (P<0.05) and reached 23.50%. In this group, the positive contribution of varicocele surgery to sperm ANAE positivity was evident. Although it was slightly lower than that of the non-smoking varicocele surgery group, the difference between the mean values of the two groups was not statistically significant (P >0.05, Figure 5).

While the ACP-ase positivity was the highest (38.65%) in the non-smoker and non-varicocele group, the smoker-varicocele group had the lowest ACP-ase positivity (4.82%). ACP-ase positivity in the non-smoking varicocele group was significantly (P<0.05) lower than the non-smoking non-varicocele group (Figure 5). ACP-ase positivity increased significantly (P<0.05) and reached 24.95% in the non-smoking group and varicocelectomy group. Although ACP-ase positivity increased significantly (P<0.05) and reached 22.93% in the smoker varicocelectomy group, the positivity was slightly lower (24.95%) than in the non-smoker varicocelectomy group (Figure 5).

ALP-ase positivity was highest (38.95%) in the non-smoker non-varicocele group, it was lowest (3.98%) in the smoker varicocele group. The positivity of the non-smoker with varicocele group was significantly (P<0.05) lower (11.94%) than the non-smoker non-varicocele group (Figure 4). ALP-ase positivity increased (25.21%) in the non-smoking with varicocelectomy group. Although ALP-ase positivity increased (24.85%) in the smoker with varicocelectomy group, the increase was slightly lower than that of the non-smoker with varicocelectomy group. The difference between the mean values of the two groups was statistically insignificant (P>0.05, Figure 5).

Discussion

Semen quality evaluation is the first and most important step in determining male infertility. In previous years, sperm concentration, vitality, motility, and morphological features that can be examined with light microscope gathered attentions in determining semen quality [22]. However these parameters are relatively far from determining the actual fertilization capacity of the sperm. Reactive oxygen species (ROS) induce formation of malondialdehyde, a known marker of lipid peroxidation from oxidative injury. Such oxidative modifications result in severe detoriations in sperm lipid membranes, leading to suboptimal fertilization [23]. Fertilizing potential of sperm mainly depends on the integrity and functionality all of the cellular structures, especially plasma membrane integrity [23,24]. Determination of acrosomal membrane integrity is of great importance in this respect. One of the simplest techniques that can be applied for this purpose is the determination of sperm enzymes, especially acrosomal enzymes. Due to the disruption of sperm production stages, these enzymes are either produced insufficiently or leak into the seminal plasma due to membrane damage. In this case, the acrosome reaction does not occur normally and the sperm becomes infertile. Therefore, determining the leakage of sperm acrosomal enzymes into the seminal plasma can provide important information about the acrosomal and cell membrane integrity of the sperm [11]. Thus, cytochemical analysis of sperm enzymes, especially acrosomal enzymes, can ensure important findings in the evaluation of semen quality.

Scientific data regarding the harmful effects of smoking and varicocele on sperm enzymes are not sufficient. In this study, the positivity patterns of ANAE, ACP-ase and ALP-ase showed similar properties in all groups**.** ANAE, ACP-ase and ALP-ase positivity levels of the non-smoking varicocele group were significantly (P<0.05) lower than the non-smoking non-varicocele group. These results show that varicocele has significant negative effects on the ANAE, ACP-ase and ALP-ase positivity levels of sperm. Indeed, the positivity levels of the investigated enzymes were significantly higher (P<0.05) in the non-smoker with varicocelectomy group. This increase shows that varicocele surgery improves testicular thermoregulation and increases the positivity of the examined enzymes. Sperm enzymes play an important role in sperm function, especially fertilization. Acrosomal enzymes facilitate the penetration of sperm into oocyte II by ensuring the dispersion of cumulus oophorus and corona radiata cells and the breakdown of hyaluron-

ic acid in the zona pellucida during fertilization. Wislocki [14] reported that ACP-ase and ALP-ase were found in human sperm. ACP-ase is mainly found in the head, equatorial segment, galea capitis, middle and tail pieces of human sperm. The ALP-ase reaction was mainly seen at the head and neck junction [16]. In this study, ACP-ase and ALP-ase showed a positivity pattern similar to the findings of previous researchers [14-16]. Both ACP-ase and ALP-ase are lysosomal enzymes. ACP-ase is obtained at the last stage of spermatogenesis and regulates the Ca+2 ion level of the microenvironment by catalyzing the hydrolysis of various phosphate esters [24]. Because ACP-ase and ALP-ase are found in high concentrations in the prostate, these enzymes are the most important seminal plasma enzymes and their levels in seminal plasma can provide valuable information about the health and function of the prostate.

In men with varicocele, the variables of conventional semen analysis showed greater relations to sperm DNA quality, mitochondrial activity and acrosome integrity [22]. Acrosomal enzymes are found in seminal plasma at different concentrations depending on the permeability of the acrosomal membranes and the degree of damage to the membranes. Since the integrity of acrosomal membranes is extremely important in preventing premature leakage of acrosomal enzymes, membrane disruption is one of the earliest morphological signs of spermatozoon damage. A damaged sperm severely loses its penetration and fertilization ability. Thus, the integrity of the acrosomal membranes has vital importance in spermatozoon penetration. Previous researchers suggested that there might be a close relationship between the increased enzymatic activity of seminal plasma and poor semen fertility [25,26]. Cytochemical staining of sperm enzymes in semen smears can be an important tool in determining damage to sperm membranes. In a previous study [11], ANAE positivity significantly decreased and extracelular enzyme positivity was also observed between sperm culsters in frozen-thawed bull semen. The researchers [11] concluded that these results may be due to the membrane damage occurring during freeze-thaw processes. Significant declines in ANAE positivity in the tobacco smokers with varicocele group of the present study might show that varicocele and smoking may cause structural defects in sperm membranes. Similarly, the low positivity rates of ACP-ase and ALP-ase in the smokers with varicocele group also supports the result reported above. High total acrosin activity has been reported in varicocele individuals [27]. Their results revealed that DNA fragmentation and protamine deficiency, as negative parameters in fertility, improved post-surgery; however, total acrosin activity as a positive parameter in fertility is higher in the varicocele individuals compared with fertile and decreases to a value close to the fertile control post-surgery.

Varicocele is the cause for one-third of all cases of male infertility [2]. In a varicocele, intrascrotal temperature increases and causes abnormal testicular histology, and decreases sperm quality [28,29]. Varicocele also negatively affects sperm function by disrupting the acrosome reaction [29]. Sperm motility reduces, possibly due to the production of sperm with structural and functional defects. In the present study, the non-smokers group with varicocele had significantly lower ($P<0.05$) sperm concentrations than the non-smokers group without varicocele. Varicocele surgery resulted in a significant recovery in sperm concentration. This result evidenced that varicocele hinders sperm production.

Varicocele reduces testicular blood renewal with a consequent accumulation of toxic substances. Thus, it can potentiate the toxic effects of environmental exposure to genotoxic substances such as those found in cigarette smoke [4,30]. In men with varicocele, smoking is associated with altered semen quality, decreased sperm functional integrity and seminal oxidative stress. Alterations in seminal plasma protein profiles are also present and may explain the altered semen phenotype [4]. In the present study, the sperm concentration and sperm motility decreased significantly when tobacco smoking and varicocele coexisted. Smoking significantly reduced the positive effect of varicocele surgery. Non-smoking and the absence of varicocele ensure that sperm motility is normal. Although varicocele surgery significantly increased sperm motility, continue smoking reduced the positive effect of varicocele surgery on sperm motility.

Conclusions

The enzyme cytochemical results of this study have revealed that smoking and varicocele caused signifi-

cant decreases in sperm counts and motility in accordance with the decreases in sperm ANAE, ACP-ase, and ALP-ase positivity levels. Varicocelectomy provided a significant improvement in the positivity of these enzymes. Nevertheless, keeping smoking resulted in a significant decrease in the positive effect of the surgery. Although this study covered a small number of samples, the cytochemially demonstrated ANAE, ACP-ase, and ALP-ase positivity level of spermatozoa can be considered as an auxiliary parameter in the evaluation of semen quality in humans.

Declarations

Ethics Approval and Consent to Participate

The Ethics Committee of Selçuk University Medical Faculty approved the study (Decision number: 2017/187, date: 07.6.2017). All methods were performed in accordance with the relevant guidelines and regulations. All participants signed informed consent.

Consent for Publication

Consent for publication was acquired from all patients.

Availability of Data and Material

All data generated and/ or analysed during this study are included in this published article.

Competing Interests

The authors have no competing interests.

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Authors' Contributions

RE and İÇ contributed to conception and study design; RE and ÜNC contributed to data collection and laboratory investigations; İÇ analysed the data; RE and ÜNC drafted the initial manuscript; RE, İÇ and ÜNC reviewed and approved the final manuscript.

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